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Host range of *Ceutorhynchus assimilis* (Coleoptera: Curculionidae), a candidate for biological control of *Lepidium draba* (Brassicaceae) in the USA

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Abstract

Ceutorhynchus assimilis has been selected as a potential biological control agent of *Lepidium draba*, which is a Eurasian invasive weed in North America. Preliminary studies indicated specificity of this weevil collected in southern France on *L. draba.* This result was in discord with the pest status of *C. assimilis* found in the literature. Host-specificity tests based both on field and laboratory experiments showed heterogeneity in the host spectrum of the weevils reared from different host-plants as determined by larval development. However, no distinguishable morphological differences could be visually detected between the populations feeding on different host-plants. All sampled populations of weevils were polyphagous as adults. Weevils reared from *L. draba* were specific to this plant for their complete larval development. Conversely, populations living on other wild and cultivated Brassicaceae species were not able to use *L. draba* as a host plant. Such differentiation is further highlighted by other biological aspects such as plant infestation rates, sex-ratio, duration of larval development, and differences in the timing of their life cycles. These results demonstrate that *C. assimilis*, an insect species formerly considered as a pest of Brassicaceae, is characterized by its host-range variability, with one population being potentially useful in the biological control of *L. draba*. Moreover, this example points to the need to test multiple populations of biological control agents in assessing risk.

Keywords: Weed biological control; Morphocryptic entities; *Lepidium draba*; *Ceutorhynchus assimilis*; Host range; Risk assessment

1. Introduction

Heart-podded hoary cress, *Lepidium draba* L. (= *Lepidium draba* subsp. *draba* L.) (Brassicaceae) probably originated from central Asia and currently occurs in most temperate areas of the world, including the United States (Mulligan and Findlay, 1974). This weed is well adapted to moist habitats, especially subirrigated pastures, rangelands, roadsides, and ditch banks. It is toxic to cattle because of glucosinolate secondary compounds and negatively impacts forage production. It also competes with native plant species, drastically reducing the local biodiversity (Sheley and Stivers, 1999). Success of this weed is directly related to its great capacity for vegetative reproduction by deep rhizomes and its prolific seed production and dispersion (Mulligan and Findlay, 1974). The use of herbicides to control this weed, which is not always feasible, also affects nontarget plants, causes disturbance in the ecological balance, and pollutes land and water resources. These limitations have motivated an investigation of alternative approaches, such as biological control by using natural enemies. Among the insects occurring on *L. draba*, a few seem specific to this weed (Lipa, 1974),

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and potentially useful in a biological control framework. The only candidate found so far is a mite, *Aceria draba* Nal. (Acari: Eriophidae) that specifically attacks the flowers of heart-podded hoary cress (Sobhian et al., unpublished data). Given that successful biological control strategies should integrate complexes of auxiliary species with potential complementary effects, there is a need for prospecting for other natural enemies (Denoth et al., 2002).

Recently, a collar gall-weevil, *Ceutorhynchus assimilis* (Paykull, 1792) [= *Ceuthorrhynchus pleurostigma* (Marsham, 1802) syn.n.] (Coleoptera: Curculionidae) (Colonnelli, 1993), has been recognized as a potential biological control agent (Fumanal et al., 2000). Indeed, according to Harris and Shorthouse (1996), gall-making insects generally display high specificity to their hosts due to strong interactions with the plant, and are considered as efficient biological control agents of weeds. *C. assimilis* is widely distributed in Eurasia and Northern Africa (Dennis, 1987). The adult weevil is characterized by white or yellow lateral spots on its thoraco-elytral angle (Hoffmann, 1954; Perrier, 1961). *C. assimilis* might have one, two, or more egg-laying periods per year (Jourdheuil, 1963). Larvae develop in galls formed at the collar of the plant whereas the pupal stage occurs in the soil (Jourdheuil, 1963).

Despite numerous taxonomic revisions of the genus (Colonnelli, 1993; Hoffmann, 1954; Tempère, 1975; Tempère and Péricart, 1989), there is no detailed description or studies of the host range within the *Ceutorhynchus* genus. Hoffmann (1954) listed this weevil as a pest of more than 13 species of Brassicaceae including several crops. Until now, this statement has prevented consideration of using *C. assimilis* as a potential biological control agent for heart-podded hoary cress. However, preliminary observations and host-choice tests showed that populations from southern France (currently identified as belonging to *C. assimilis*) reared from galls on *L. draba* displayed at least a marked preference for this host both for egg-laying and larval development (Fumanal et al., 2000). These data suggest the need for reevaluating the polyphagous status of the weevil.

In this paper, we evaluate the host range of *C. assimilis* using host-choice and no-choice testing procedures in the field and laboratory following the classical procedures (Marohasy, 1998; Wapshere, 1975). We examine the host range of some weevil populations found on various plant species from different localities in Western Europe. The study had three objectives: (i) to understand the field host uses and biology of *C. assimilis*; (ii) develop hypotheses to accommodate the observed pattern of host specificity; and (iii) to discuss the implications of this knowledge for the development of the biological control program against *L. draba*.

2. Material and methods

2.1. Host-specificity tests

The host range of *C. assimilis* was determined from oviposition and successful gall development on various plants including the target weed, *L. draba*, and by measuring the rate of successful development of the larvae inside the galls.

2.1.1. Plant material

Emphasis in the selection of host-plants was given to species closely related to *L. draba* within the Brassicaceae, focusing on plants that were previously recorded as hosts, including crops of economic importance. The plants used in the different experiments are listed in Table 1. The plants were grown from seeds in individual pots (1 L), each filled with sterile soil and kept in a greenhouse at 25 °C under natural lighting. After 1 month, when the plants reached optimum size, they were transferred to the field plot, or maintained in the cages for the laboratory tests.

2.1.2. Insects used in the laboratory tests

Samples of *C. assimilis* populations used for the different tests were identified as belonging to the same

Table 1
Scientific and common names of the Brassicaceae plants used in the field and laboratory tests

Tested plants		Field test and laboratory tests I and II	Laboratory tests III–VI
Species name	Common name		
Brassica campestris L. var. *rapa* (Metzg.) Sink.	Turnip	x	x
Brassica napus L. subsp. *oleifera* DC.	Rape	x	x
Brassica napus L. subsp. *rapifera* Metzger	Swedish turnip	x	
Brassica oleracea L. var. *capitata* L.	Cabbage	x	x
Lepidium draba L. Desv.	Heart-podded hoary cress	x	x
Diplotaxis erucoïdes DC.	White wall-rocket		x
Lepidium latifolium L.	Perennial pepperweed		x
Raphanus sativus L.	Radish	x	x
Sinapis arvensis L.	Wild mustard		x

morphological form (E. Colonnelli, University of Rome, Rome, Italy, personal communication, 2000, 2002). Mature larvae were extracted from galls on different host plants and at different localities (Table 2). For each population tested, a total of 100 larvae were put in moist sterile soil contained in small plastic boxes (10-cm diameter). The larvae pupated at 20 °C and 70% relative humidity (RH), at 1–5 cm below the soil surface for 25–30 days. The emerging adults were then placed on host plants for feeding. The adults were able to oviposit both after the summer diapause in early September or directly in May when they were collected at the beginning of spring.

2.1.3. Insects used in the field test

The insects used in the field experiment were adults of *C. assimilis* which emerged from *L. draba* in May and June, and were locally present after summer diapause where the field plot was set up (Table 2).

2.1.4. Field experiment

A field plot (15×5 m) was established in September 2000 at the European Biological Control Laboratory (Montferrier sur Lez, France), within the native range of the local population of *C. assimilis* occurring on *L. draba*. The experiment was set up as a randomized complete block design with 15 replicates. A total of 90 plants (6 species $\times$ 15 replicates as listed in Table 1) were planted 1 m apart (6 rows $\times$ 15 columns). A total of 120 *L. draba* plants naturally infested by *C. assimilis* were collected from a nearby population and transplanted around the plot to increase the local population of the insect on the test (20 *L. draba* disposed in 6 groups). The ability of *C. assimilis* to develop both galls and larvae on plants (= infestation rate) was checked every month for 5 months, then the plants were dissected. Descriptive statistics of the data were based on the presence or absence of galls on plants and on the mortality of host-plants. Correlation between data sets was assessed using Pearson's rank correlation.

2.1.5. Laboratory tests

The ability of *C. assimilis* adults to feed and develop both galls and larvae on a large range of plants was also tested under controlled conditions in small-cage experiments for three consecutive years (2000, 2001, and 2002). These experiments (Table 2) included both choice tests (i.e., all tested plant species were randomly mixed in a cage) and no-choice tests (i.e., all tested plant species were isolated in individual cages) and were carried out with the same plant species as listed in Table 1. The tests were conducted with slightly different designs regarding the number of replicates and plant species tested as summarized in Table 2. We investigated the host range of weevil populations living on *L. draba* (tests I, II, and III) and complemented this approach by testing three other *C. assimilis* populations, respectively from *Brassica napus* L. subsp. *oleifera* DC. (IV), *Brassica oleracea* L. var. *capitata* L. (V), and *Sinapis arvensis* L. (VI) (Table 2). The plants were randomly arranged in a closed mesh-cage ($100 \times 80 \times 70$ cm, width $\times$ depth $\times$ height front to back) for choice tests, whereas single plants were placed in individual smaller cages ($10 \times 10 \times 50$ cm) for no-choice tests. A total of 40 adult weevils (20 males and 20 females) were reared and introduced into each choice test, while four weevils (2 males and females) were used for no-choice tests. Experiments were conducted in a greenhouse under natural light conditions from September to January for experiments I and II and from March to July for experiments III to VI. The mean temperature ranged from 23.3 ± 0.3 ($\pm$SE) to 19.6 ± 7.9 with mean RH from $63.6\% \pm 5.2$ to $63\% \pm 24$.

Adult feeding on plant leaves was estimated for all the tests (except test II), 2 weeks after the introduction of the weevils into the cages. Oviposition, abortion, or gall formation were also recorded but at different times during the tests. At the end of the test (5 months later), galls were dissected to check for complete larval development to adult emergence (verified by subsequent rearing). Damage to leaves by adults was assessed using a leaf-damage assessment index ranging from 0 to 3 based

Table 2
Experiments used for the evaluation of the host specificity of *C. assimilis* using field choice test and choice and no choice laboratory tests from 2000 to 2002

Test	Host-plant species	Site location	Site GPS	No. of replicates
Field	*Lepidium draba*	Montferrier sur Lez, Hérault, France	43°41′11″ N 03°52′19″ E	15
Laboratory I	*Lepidium draba*	Carnon, Hérault, France	43°36′54″ N 04°00′36″ E	2
Laboratory II	*Lepidium draba*	Montferrier sur Lez, Hérault, France	43°41′11″ N 03°52′19″ E	3
Laboratory III	*Lepidium draba*	Montferrier sur Lez, Hérault, France	43°41′11″ N 03°52′19″ E	4
Laboratory IV	*Brassica napus* subsp. *oleifera*	Cherves, Deux-Sèvres, France	46°21′32″ N 00°33′41″ E	4
Laboratory V	*Brassica oleracea* var. *capitata*	Oulmes, Deux-Sèvres, France	46°21′52″ N 00°27′55″ E	4
Laboratory VI	*Sinapis arvensis*	Assas, Hérault, France	43°40′02″ N 03°54′12″ E	4

on total leaf surface eaten (0: no damage, 1: 0–10% damaged, 2: 10–20% damaged, and 3: 20–30% damaged) and related to the developmental stage of the plant.

Data from tests I and II were subjected to analysis of variance (ANOVA one factor or ANOVA two factors crosses without repetitions) or the nonparametric test of Kruskal–Wallis. The tests were performed using STATISTICA 5.0 software (StatSoft, 1995). For the evaluation of larval development, presence or absence of galls, galling rate, and numbers of larvae and adults were recorded. Data were analyzed using the Student mean-comparison test, when assumptions of normality and homogeneity of variance were satisfied and with nonparametric tests of Mann–Whitney and Kruskal–Wallis (Zar, 1999).

2.2. Biological observations

The data on the biology and behavior of *C. assimilis* were collected from observations during field surveys on various host plants and areas and host-specificity and rearing experiments. Data collected were: (i) the infestation rate as expressed by the percentage of infested plants out of 100 random samples per locality; (ii) rate of hymenopteran endoparasitism, duration of the pupating period, and sex-ratios of reared weevils; and (iii) the life cycles of the weevils from the three populations from southern France were compared (two of these corresponding to populations used in host specificity tests II, III, and VI, Table 2). We sampled sympatric populations living on *L. draba* (population A) and *S. arvensis* L. (population B, used in test VI) from the same site to investigate the variability of the life cycle in relation to the host-plant species. We also sampled a third population (C) on *L. draba* but in a different site (corresponding to tests II and III, Table 2) to observe whether the observed variability was due to a host-plant or site effect. For each population, 30 galled plants were dissected each month from November 2002 to April 2003. The number of larvae at the different stages was recorded (L1, L2, and L3), as well as the number of exit holes caused by larvae seen in galls. Duration of larval stages was calculated and characterized by measuring head-capsule widths. We computed descriptive statistics of the data and proportions were compared using a Z test or t test.

3. Results

3.1. Host-specificity tests

3.1.1. Field test

Infestation rates by *C. assimilis* and mortality of the test plants are shown in Fig. 1. The cephalic capsule widths (CCW) for the different larval instars were as follows ($n = 20$): L1 0.17 ± 0.01, L2 0.37 ± 0.02, and L3 0.60 ± 0.02 mm. All *L. draba* plants displayed galls at the end of the experiment, whereas no gall symptoms were found on any other test plant species. The total number of larvae per infested *L. draba* plant averaged 3.8 ± 1.1 ($\pm$SE).

Plant mortality rates ranged from 60% for *L. draba* infested by the weevil to an average of 12% for the other Brassicaeae species not infested (maximum of 20% for *B. oleraceae*). Weevil infestation rate was strongly correlated with host-plant mortality ($r = 0.97$; $P = 0.001$). The mortality rates of non-infested plants and their variability may be explained by environmental constraints.

3.1.2. Laboratory testing—adult feeding

Adult feeding was observed on leaves for all plant species in choice test I (Fig. 2). In no-choice test I, feeding was restricted to four test species (*L. draba*, *Brassica campestris* var. *rapa*, *B. oleracea*, and *Raphanus sativus*). In both tests, damage due to adult feeding was in general heterogeneous and in particular always higher

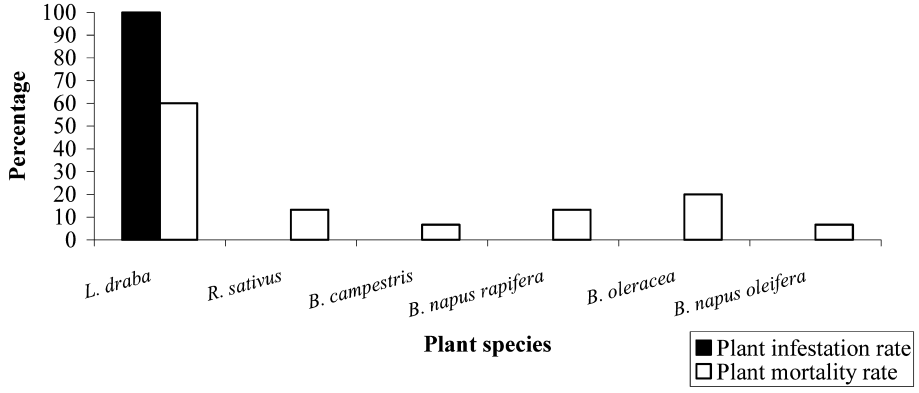

Fig. 1. Plant mortality and infestation rates on six host-plant species by the gall-making weevil *C. assimilis* during the open field test with natural populations.

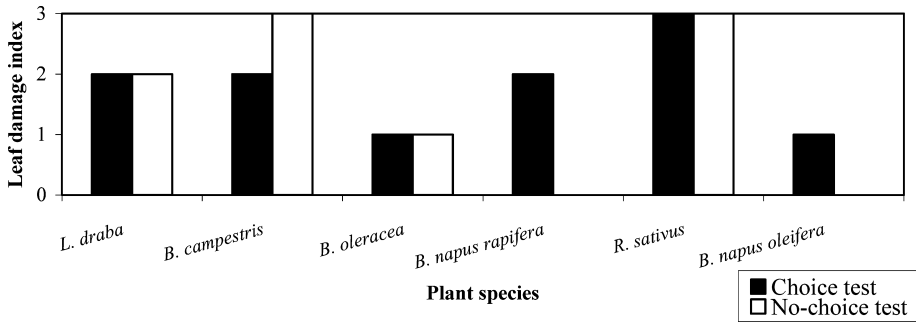

Fig. 2. Adult *C. assimilis* feeding on six host-plant species in choice and no-choice tests I. Leaf damage was assessed using values ranging from 0 to 3 based on percentage of leaf area eaten and related to the developmental stage of the plant. Adult feeding was considered as high (3) when 20–30% of total plant leaf surfaces were damaged by the weevil.

on *R. sativus* than on the control *L. draba*. A similar pattern of polyphagy was obtained from the other four experiments for which data were collected but with some variations between populations tested (III, IV, V, and VI). In these tests, a significant difference in adult feeding was observed among the different weevil populations including choice and no-choice tests regardless of host-plants ($P = 0.011$). Multiple comparison procedures (Dunn's method), showed significant differences in adult feeding between populations V/III and V/IV. We observed traces of adult feeding on all the plant species, but feeding patterns differed between choice and no-choice tests, depending on the test species. ANOVA of adult feeding between host-plants based on the mean index of leaf-damage (Table 3) indicated no difference within choice and no-choice tests. However, more adult feeding in choice tests than in no-choice tests was observed, which was probably due to the higher insect pressure, or may be related to the presence of the original host plant in the choice tests. The main result is polyphagous adult feeding. There is no general correlation between the level of damage and the origin of weevil populations, but insects reared from *L. draba*, *B. napus*, and *B. oleracea* tended to feed less on their original hosts than on other plants. Indeed, within test III (choice test), the mean leaf damage on the natural host-plant *L. draba* (0.75 ± 0.43) was lower than the mean damage of the test (all plants combined) (0.97 ± 0.2), whereas there was a preference for the other wild plants such as *S. arvensis*, and *Diplotaxis erucoïdes* DC. ($P = 0.03$).

3.1.3. Laboratory testing—larval development

In tests I and II (for which *L. draba* is the natural host-plant), larval development was completed only on *L. draba* (Table 4) although the mean number of eggs laid was very high on other host-plants (Test I, 16.75 ± 6.74 eggs). In contrast with the field experiment, we also found evidence for larval development to first instars on other Brassicaceae (*B. campestris* and *R. sativus* in the choice tests and both on *B. campestris* and *B. oleracea* in the no-choice tests). The absence of complete gall formation and failed larval development are correlated, but the mechanism of such a pattern is not clear. Development of the larvae from the different insect populations, in tests III–VI (Table 4), showed other patterns. The mean number of emerging adults was used to assess a capacity to complete the life cycle on the host-plant. Population VI showed no larval development and adult emergence even from the native host (*S. arvensis*) and so was ignored. Insects from population III, reared from *L. draba*, completed larval development only on this plant (Table 4). Gall development appeared on *R.*

Table 3
Analysis of variance with two factors of adult feeding, based on key index of leaf damage, for the four laboratory specificity tests (III–VI)[a]

Test	Comparison between choice (c) and no-choice (nc) tests (probability, mean, and standard error)		Comparison between host plants attacked by the test type, choice, and no-choice
III	$^{*}P < 0.001$	c 0.969 ± 0.124 nc 2.156 ± 0.124	$^{*}P < 0.001$
IV	$^{*}P = 0.005$	c 1.219 ± 0.148 nc 1.844 ± 0.148	$P = 0.189$
V	$^{*}P < 0.001$	c 1.647 ± 0.140 nc 2.464 ± 0.136	$^{*}P = 0.022$
VI	$^{*}P = 0.031$	c 1.375 ± 0.118 nc 1.179 ± 8.143	$P = 0.086$

[a] Comparisons are made between choice and no-choice tests and different host plant species attacked.
[*] Significant difference for $\alpha = 0.05$.

Table 4
Larval development in tests I–V for plants with positive larval development, presence of galls, larvae and adults, and F1 adults alone[a,b]

Test	Population host plant origin	Modality of test	Host plant infested	Number of parent weevils	Number of replicates with larval development/total number of replicates	Gall mean number/ species	Larva and adult mean number	Total F1 adults
I	*L. draba*	Choice	*B. campestris*	40	1 of 2	1	1	0
I	*L. draba*	Choice	*L. draba*	40	1 of 2	1	1	1
I	*L. draba*	No-choice	*B. campestris*	4	1 of 2	1	1	0
I	*L. draba*	No-choice	*L. draba*	4	2 of 2	1.5 (±0.71)	1.5 (±0.71)	3
II	*L. draba*	Choice	*R. sativus*	40	2 of 3	5 (±1.41)	6 (±1.42)	0
II	*L. draba*	Choice	*L. draba*	40	3 of 3	1.3 (±0.58)	1.7 (±0.58)	4
II	*L. draba*	No-choice	*B. oleracea*	4	1 of 3	1	1	0
II	*L. draba*	No-choice	*L. draba*	4	2 of 3	2	2	4
III	*L. draba*	Choice	*R. sativus*	40	0 of 4	7.67 (±2.08)	0	0
III	*L. draba*	Choice	*L. draba*	40	3 of 4	20.33 (±12.34)	14 (±11.27)	2
III	*L. draba*	No-choice	*L. draba*	4	2 of 4	14 (±12.72)	17	10
IV	*B. napus oleifera*	Choice	*D. erucoïde s*	40	1 of 4	3	0	0
IV	*B. napus oleifera*	Choice	*B. campestris*	40	4 of 4	35 (±15.72)	28.33 (±17.24)	17
IV	*B. napus oleifera*	Choice	*B. oleracea*	40	3 of 4	3.67 (±1.15)	4 (±2)	0
IV	*B. napus oleifera*	Choice	*B. napus oleifera*	40	2 of 4	5.5 (±4.95)	1	0
IV	*B. napus oleifera*	No-choice	*B. napus oleifera*	4	3 of 4	18 (±14)	8 (±8.18)	8
V	*B. oleracea*	Choice	*B. oleracea*	40	2 of 4	1	0.5 (±0.71)	0
V	*B. oleracea*	Choice	*B. napus oleifera*	40	4 of 4	6 (±2.16)	1.75 (±1.71)	3
V	*B. oleracea*	No-choice	*B. napus oleifera*	4	3 of 4	15.67 (±11.51)	9 (±10.15)	14

[a] No data available for test VI because no larval development was obtained.
[b] Calculation of means is based on infested plant replicates, total numbers of F1 adults indicates the complete development on the host plant.

sativus in the choice test, but larval development failed early on. The weevil population reared from *Brassica napus oleifera* (IV) completed larval development on four host plants under choice conditions [with more larvae and adults on *B. campestris* (mean: 28.33 ± 17.24), than on the natural host plants (mean: 2.5 ± 0.71)], but completed development only on its natural host-plant in no-choice tests (Table 4). Finally, the population reared from *B. oleracea* (V) showed larval development on *B. oleracea* and *B. napus oleifera* in the choice test but only on *B. napus oleifera* in the no-choice test (Table 4).

Comparisons between tests showed that there is neither significant difference in larval development (or gall formation), nor in larval numbers between the different host plants under choice conditions (for $\alpha = 0.05$) or between test types (choice and no choice) ($P = 0.14$). The potential egg-laying, based on gall numbers, is homogeneous, i.e., no significant differences ($P = 0.73$) among the three tests III, IV, and V.

3.2. Additional biological observations

3.2.1. Infestation rate

Field observations showed considerable spatial variation in the occurrence of the natural infestation for a given host plant. Infestation rates of *C. assimilis*, which were measured by larvae and gall presence on *L. draba*, in southern France, Northern Italy, and Spain, were high (50–92%) compared to populations found on other wild plants such as *S. arvensis* (10%) or *D. erucoïdes* (4%) in the same regions. Populations of *C. assimilis* observed on crop plants were quite limited in some areas of Western France (*B. napus oleifera* and *B. oleracea*), Northern Austria (*B. napus oleifera*), and Central Italy (*B. oleracea*) and in highly variable proportions for a given area (2–62% in Western France on *B. napus oleifera*). Moreover, in Western France, the infestation rates were very low on other wild Brassicaceae (*S. arvensis*: 1.6%).

3.2.2. Sex-ratio, pupation duration, and larval parasitism

Data collected during the rearing of weevil populations (from different host-plants listed in Table 2) provided evidence of biological differentiation. Sex-ratio comparisons using the Z test (taking into account the population size) showed that populations IV from *B. napus oleifera* (55% males and 45% females), V from *B. oleracea* (59% males and 41% females), and VI from *S. arvensis* (57% males and 43% females) were not different from each other. Although, this group of populations was significantly more male-biased than population III from *L. draba* (39% males and 61% females) ($P < 0.005$). Also, the duration of pupal stage was shorter for populations from *L. draba* (29.2 days) than for the three other populations (from 31.5 to 32 days).

Furthermore, endoparasitism rates were lower for populations from *L. draba* (1%) than for the other populations (4–14%).

3.2.3. Life cycle

The sympatric weevil populations developing on *L. draba* (A) developed faster than that on *S. arvensis* (B) at the same site (Fig. 3). In November, first larval stages (L1) were predominant in comparison with the mature stage (L3) in population B, as opposed to population A. This suggests that oviposition occurs later on *S. arvensis* (B) than on *L. draba*. This pattern seems to be confirmed by an increasing numbers of larvae on *S. arvensis* from November to January (Fig. 3) followed by a rapid decrease until April in contrast to the continuous decrease of the populations living on *L. draba* (A) from November to April. The decline in the number of larvae was directly related to the increasing number of exit-holes from galls. Larvae from all populations completed their development in April at the latest. More precisely, the oviposition time on *S. arvensis* seems to be both delayed and extended in time compared to those on *L. draba* which displayed a short oviposition window. Moreover, the population on *L. draba* is able to display continuous larval pupation (and a potentially small but continuous new adult emergence during winter), compared with a short emergence peak of *S. arvensis* in spring.

In contrast, there was no difference in larval development between populations A (*L. draba*) and C (*L. draba*) from different locations (L1 $P = 0.62$; L2 $P = 0.47$; L3 $P = 0.23$; Exit holes $P = 0.74$) using the t test comparison. The comparison of total larval frequencies for the three populations (using Pearson's product moment correlation) indicated a significant correlation for populations on *L. draba* (A/C $P = 0.002$) and no correlation between *L. draba* and *S. arvensis* populations (A/B $P = 0.26$; C/B $P = 0.31$). These results suggest that the variability observed between populations A and B is not due to site variation but probably to host-plant difference.

4. Discussion

The collar root-gall weevil *C. assimilis* has been reported to attack a number of genera and species in the Brassicaceae, to such an extent that this insect species is thought to be a pest on cabbage and other cultivated plants (Hoffmann, 1954; Jourdheuil, 1963). The results presented here clearly suggest that the true situation is more complicated than this. Both field studies and host-specificity tests show that, within the species *C. assimilis*, there are at least two groups in southern France. The first one is on *L. draba* and appears to have a separate phenology and larvae

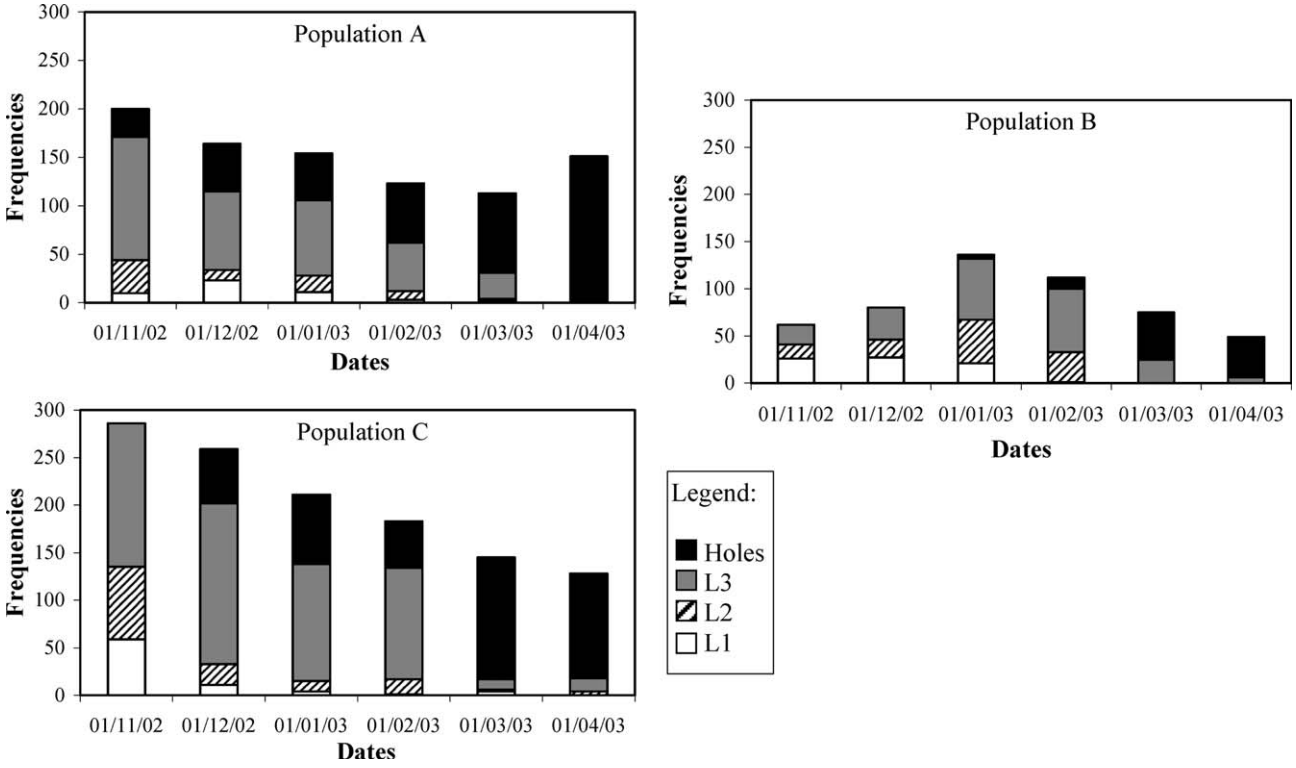

Fig. 3. Three life cycles of *C. assimilis* populations developing on different host plants and different sites in southern France (population A from *L. draba*, site IV; population B from *S. arvensis*, site IV; and population C from *L. draba*, sites II and III). The frequencies of eggs, larval stages (L1, L2, and L3), and holes on galls caused by mature larvae leaving for pupation were monitored for 6 months.

which develop only on this plant and a second group that appears to consist of more generalists in nature. Our results also confirmed the polyphagous character of the weevil adults feeding for all the populations tested but, at the same time, highlighted differing performances of the larvae with respect to the host-plant from which the adults were collected. In this respect, as suggested by Briese (1998), the concordance between our results obtained from both field and laboratory tests provide strong support for the validation of the insect's host-range.

Van Klinken (2000) argues that the fundamental host range for biological control can be described separately for oviposition, egg development, larval development, and adult feeding. Furthermore, concerning adult feeding in laboratory tests, if there is no maturation of eggs or complete larval development on a nontarget plant, there is a tendency to discount any adult feeding which does occur (Cullen, 1989). In fact, polyphagy in adult feeding is not of primary importance as this may lead to only superficial damage to nontarget plants. Therefore, other biological criteria, and in particular larval development, should be taken into account in the evaluation of host specificity (Briese et al., 2002; Jourdheuil, 1963). In the case of *C. assimilis*, the pattern of larval development is much more varied than the adult feeding. Indeed, only weevils reared from *L. draba* completed their larval development on this weed, and these larvae failed to complete their development on other host-plants. Conversely, insects reared from other plants in Brassicaceae used a wide range of hosts excluding *L. draba*.

Even though the mechanisms or evolutionary contingencies that led to this pattern of host use are still unclear, some aspects of the biology of *C. assimilis* provide valuable clues. In particular, for weevil populations reared from *L. draba*, we found only death of first instar larvae, or eggs on other host plants. According to Cullen (1989), such results should be viewed with caution as they may result from laboratory test conditions causing indiscriminate oviposition by the adults. In line with this, we have shown that the oviposition rate was always higher in the choice tests than in no-choice tests. This tendency was explained by Sands and Van Driesche (2000) as resulting from the presence of the natural host-plant inducing abnormal oviposition behavior. In any case, a lack of complete larval development is a key factor in host specificity evaluation. According to Mattson et al. (1987) the insect gall-makers have intimate relationships with their host plant and are therefore sensitive to variations in plant characteristics.

Two mechanisms may explain the failure to induce galls on some potential hosts. First, the plant may produce specific defense compounds that prevent larval development. The ability of insects to accommodate secondary compounds involved in plant defense can explain the specialization for a particular host (Futuyma, 2000), involving a tradeoff between specialization and general adaptation. This is in agreement with some hypotheses (as reviewed in Jourdheuil, 1963) concerning the gall formation process for *C. assimilis*, which proposes that gall induction may be provoked by teratogenic substances inoculated by the adults during oviposition or by hormonal substances derived from within the body of the larvae.

Second, some authors (Crook et al., 2001; Mattson et al., 1987) suggested that plant species, other than the natural host plant, may have unsuitable morphology that prevents gall formation. According to Ananthakrishnan (1984), initiation and exploitation of plant tissues leading to the formation of plant galls by insects is considered as a highly developed form of association. The host plant, through the gall formation process, has possibly compelled the gall-maker to become an extremely specialized feeder (Mani, 1964; cited in Ananthakrishnan, 1984).

The differentiation exhibited by host specificity is also found for other biological features of the weevil populations. The variability in infestation rates was influenced by the geographic origin and the species of natural host plant. For example, in southern France, the infestation rate was always higher on *L. draba* than on the other wild host plants and almost residual on crop plants. This was not the case, however, in other regions where *L. draba* is absent (Central Western France, Central Italy, and Austria). This trend might be explained by historical and ecological factors that led to the current distribution of host plants. In addition, other biological features, i.e., differences in the timing of the life cycle, inversion of sex ratio, and differential duration of the nymphal stage clearly highlight the biological differentiation of weevil populations in relation to their natural host plants.

The combination of biological data collected and analyzed in this paper clearly demonstrates that *C. assimilis* exhibits biological and ecological differentiation in host use. This discovery has strong implications for assessments of host specificity of this weevil and hence for its utility in the biological control of *L. draba*. In light of the differentiation observed, exploring the genetic structure of the weevil populations throughout its geographical range and across its known host plants in addition to *L. draba* merits further investigation. Testing the specificity of several populations carrying apparently the same morphology (E. Colonnelli, University of Rome, Rome, Italy, personal communication, 2002) but using a large spectrum of host plants should be a key point in the evaluation of *C. assimilis* as a potential biological control agent for *L. draba*. A key point of this system is the finding of both specialist and generalist forms within the same phytophagous insect, with the specialist form being closely associated with a target weed. A similar example can be found in literature

concerning the aphid pest species *Therioaphis trifolii* (Sunnucks et al., 1997). Sunnucks et al. (1997) found that this species, which is a generalist in Western Palaearctic region on alfalfa, clovers, and related legumes, displays a particular form in Australia that feeds almost exclusively on alfalfa. Moreover, they compared an other form found only on clovers and observed morphological and genetic differences between them. Such pattern of variability in the host range may be found in other insects that are currently discarded for biological control programs. The next step in the evaluation of *C. assimilis* as a potential biological control agent would undergo further testing with regard to the very large number of economically important and wild Brassicaceae in North America. A total of 66 US native or introduced plant species from 34 genera and 7 Brassicaceae tribes are listed on the plant petition list assembled by the Montana State University (Bozeman) and proposed for approval to the Technical Advisory Group (TAG) to test *C. assimilis* in the framework of biological control of *L. draba*. Indeed, only known host plants and relatives from the same tribe as *L. draba*, Lepidieae and others from the tribe Brassiceae (within Brassicaceae), including wild and economic crops, were included in this study. The current knowledge in the phylogenetic relatedness of Brassicaceae (Hall et al., 2002; Mummenhoff et al., 2001) is still in its infancy. Our results provide the prerequisite for further investigation of *C. assimilis* as a biological control agent against *L. draba*. We therefore advocate that biological control strategies and associated risk assessment should not uniquely rely on morphological identification that may mask morphocryptic entities, but instead, this criterion should be complemented by host range assessment, molecular identifications, and ecological/biological studies in the framework of host-plant association.

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